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Methane Flux Dynamics and Driving Mechanisms in Constructed Wetland

Master Thesis in Bioengineering (30 ECTS)

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Abstract

Title: Methane Flux Dynamics and Driving Mechanisms in Constructed Wetland

Methane (CH_4) is a greenhouse gas primarily produced through natural processes and human activities. Constructed wetlands (CWs), while effective for treating diffused agricultural runoff, can also emit CH_4 due to the anaerobic decomposition of organic matter. This study investigates CH_4 emissions and their driving mechanism in Vända CWs (designed to treat agricultural runoff) in Uhti village, southeast Estonia. The CWs have two sections (wetlands one and two). CH_4 emissions were measured from October 2022 to December 2023. The result reveals that both wetlands emit CH_4 emissions. However, the second wetland has higher emission with a median of 619.2 $\mu g\ m^{-2}\ hr^{-1}$ (5306.1 $\mu g\ m^{-2}\ hr^{-1}$ ± 936.5 $\mu g\ m^{-2}\ hr^{-1}$ mean ± standard error SE) and a peak emission of about 35,000 $\mu g\ m^{-2}\ hr^{-1}$ compare to the first wetland which shows a median of 218.9 $\mu g\ m^{-2}\ hr^{-1}$ (2424.4 $\mu g\ m^{-2}\ hr^{-1}$ ± 743.9 $\mu g\ m^{-2}\ hr^{-1}$) and a peak of above 15,000 $\mu g\ m^{-2}\ hr^{-1}$. Also, both wetlands exhibited seasonal variations, with summer months displaying the overall CH_4 emission with a median of 2666.8 $\mu g\ m^{-2}\ hr^{-1}$ (8393.3$\mu g\ m^{-2}\ hr^{-1}$ ± 3351.6 $\mu g\ m^{-2}\ hr^{-1}$) in the first wetland and a median of 11150.8 $\mu g\ m^{-2}\ hr^{-1}$ (19430.7 $\mu g\ m^{-2}\ hr^{-1}$ ± 3563.4 $\mu g\ m^{-2}\ hr^{-1}$) in the second wetland. Winter months showed the lowest overall CH_4 median emission of 4.9 $\mu g\ m^{-2}\ hr^{-1}$ (103.5 $\mu g\ m^{-2}\ hr^{-1}$ ±36.1 $\mu g\ m^{-2}\ hr^{-1}$) in the first wetland and a median of 4.9 $\mu g\ m^{-2}\ hr^{-1}$ (114.1 $\mu g\ m^{-2}\ hr^{-1}$ ±47.4 $\mu g\ m^{-2}\ hr^{-1}$) in the second wetland. Spearman's rank correlation analysis was used to assess the strength of the relationships between CH_4 emission and various measured parameters. The analysis revealed that several measured parameters, including dissolved oxygen (O_2), pH, oxidation-reduction potential (ORP), and conductivity, exhibited statistically significant correlations (<0.001) with CH_4 emission when compared to other parameters. Based on these findings, we recommend regular harvesting of wetland biomass and installation of aeration techniques as mitigation strategies for reducing CH_4 emissions from Vända-constructed wetlands.

Keywords: methane, constructed wetlands, emission, greenhouse gases, global warming, methanogenesis.

CERCS code: T270 Environmental technology, pollution control

Resümee

Pealkiri: Metaani vood ja seda mõjutavad tegurid vabaveelise tehismärgala näitel

Metaan (CH_4) on kasvuhoonegaas, mis tekib erinevate looduslike protsesside tulemusena peamiselt anaeroobses keskkonnas. Üks olulisemaid metaani allikaid on märgala ökosüsteemid. Vabaveelised tehismärgalad, mida kasutatakse põllumajandusliku hajukoormuse vähendamiseks võivad aja jooksul muutuda olulisteks metaani allikatest kuna pidevalt lisanduv orgaaniline aine ning anaeroobne keskkond pakub metaani tootvatele mikroobidele sobivat elukeskkonda. Käesoleva magistritöö eesmärkideks oli uurida metaani voogusid, nende tekke mehhanisme ning võimalikke meetmeid voogude vähendamiseks Vända vabaveelise tehismärgala näitel, mis asub Uhti külas Tartumaal. Märgalasüsteem koosneb kahest eraldiseisvast märgalast (esimene ja teine märgala) ning mõlemas süsteemis oli kuus proovipunkti voogude mõõtmiseks. Välitööd viidi läbi perioodil oktoober 2022 kuni detsember 2023. Tulemused kinnitasid, et mõlemad süsteemid on olulised metaaniallikad. Esimese märgala keskmine (±standardviga) metaani voog üle kogu mõõteperioodi oli oli 2424,4 µg m^2 h^{-1} ± 743,9 µg m^2 h^{-1} ning teisel märgalal 5306,1 µg m^2 h^{-1} ± 936,5 µg m^2 h^{-1}. Maksimaalsed vood suvisel perioodil ulatusid kuni 35 000 µg m^2 h^{-1}. Lisaks oli selgelt näha ka hooajaline voogude varieeruvus, kus suvekuudel oli keskmine voog oluliselt kõrgem kui talvekuudel. Näiteks esimesel märgalal oli suve keskmine voog 8393,3 µg m^2 h^{-1} ± 3351,6 µg m^2 h^{-1} ning teisel märgalal 19 430,7 µg m^2 h^{-1} ± 3563,4 µg m^2 h^{-1}. Talvine voog esimesel märgalal oli 103,5 µg m^2 h^{-1} ± 36,1 µg m^2 h^{-1} ja teises märgalas 114,1 µg m^2 h^{-1} ± 47,4 µg m^2 h^{-1}. Spearmani astakkorrelatsiooni põhjal tuli välja, et metaani voogudel oli tugev seos temperatuuri, lahustunud hapniku kontsentratsiooniga ning vee pH-ga. Töö põhjal selgus, et voogusid aitaks reguleerida tihedam kaldapealne taimkate, mis suvisel perioodi varjutamisega stabiliseeriks vee temperatuuri. Samuti aitaks voogusid vähendada perioodití taimede maapealse biomassi eemaldamine, mis vähendaks orgaanilise aine sisaldust põhjamudas.

Võtmesõnad: etaan, tehismärgala, kasvuhoonegaasid, globaalne soojenemine, metanogenees

CERCS klassifikaator: T270 keskkonnatehnoloogia, reostuskontroll

TERMS, ABBREVIATIONS AND NOTATIONS

AOM	anaerobic oxidation metal
CH_4	methane
CO_2	carbon dioxide
CWs	constructed wetlands
Fe3+	iron
FWS	free water surface
GHGs	greenhouse gases
H_2O_2	hydrogen peroxide
N_2O	nitrous oxide
O_2	oxygen
O_2^-	superoxide
ppb	parts per billion
ROS	reactive oxygen species
SF	surface flow

Contents

Introduction

Global warming is one of the significant environmental problems in the world. Globally, rising average temperatures endanger ecosystems, changing weather patterns, and increasing sea levels (Malhi et al., 2020). The main factors causing this problem are greenhouse gases (GHGs), such as methane (CH_4), nitrous oxide (N_2O), and carbon dioxide (CO_2) (IPCC, 2023). Despite these changes in weather patterns, GHG concentrations are increasing rapidly in the Earth's atmosphere and contributing to global warming (IPCC, 2023). According to Myhre et al. (2013), CH_4 alone accounts for 17% of the global radiative forcing. The atmospheric CH_4 growth rate has been high over the past decade, probably owing to the combined increases in fossil fuel and microbial sources (Turner et al., 2019). For example, CH_4 concentrations in the atmosphere rose from 720 parts per billion (ppb) in 1750, the pre-industrial level, to about 1900 ppb in 2021 (IPCC, 2023). Thus, CH_4 is thought to have warmed the planet by about 0.5 °C, compared to 0.76 °C from CO_2 and 0.24 °C from all other GHGs combined, making CH_4 the second-most significant contributor to global warming (Mar et al., 2022)

By 2050, there should be a notable decrease in GHG gas emissions to mitigate global warming by 1.5–2.0°C from pre-industrial levels (IPCC 2023). The rapid economic and industrial expansion has led to increased emissions of GHGs, raising urgent concerns about global climate change (Dreyfus et al., 2022). This urgency highlights the importance of research into strategies to reduce GHG emissions and the advancement of low-carbon technologies (Nuamah et al., 2020). Some systems that have shown an increase in CH_4 emissions are constructed wetlands (CW) (Mander et al., 2023).

CWs are engineered systems built and planned to use natural processes utilizing soils, wetland vegetation, and their associated microbial assemblages (Vymazal, 2010). The presence of vegetation, soils, and related microbial assemblages has helped treat polluted water while operating in a highly regulated setting (Vymazal, 2010). They also function as potential habitats for preserving wildlife, thereby potentially mitigating the negative impact of declining biodiversity caused by human activities, especially when applied to wildlife due to the extensive destruction of natural habitats (Hale et al., 2019). This has led to a global increase in CWs, estimated to have expanded by 5-50% since the 1970s and earlier, although precise data is challenging to ascertain due to the loss of approximately 35% of CWs data (Ramsar Convention on Wetlands, 2018). An exemplary type of CW is the free water surface (FWS) CW, characterized by areas of open water, emergent plants, and floating vegetation, either intentionally designed or as an unavoidable consequence of the design configuration (Kadlec and Wallace, 2008).

FWS CWs are characterized by areas of open water, floating vegetation, and emergent plants, either intentionally designed or as an unavoidable consequence of the design configuration (Kadlec and Wallace, 2008). There are two methods of creating surface flow (SF) constructed CWs depending on their position relative to the stream: directly into the stream (in-stream) or alongside the stream (off-stream) (Vymazal, 2010). In off-stream wetlands, only a portion of the water is directed into the CWs for purification (Punys et al., 2019). Conversely, in-stream CWs are positioned directly in the flow path, where all water from the river or ditch flows through the wetland (Kadlec and Wallace, 2008). In these SFCWs, water treatment is facilitated by the shallow water depth, low flow velocity, and presence of plant stalks and litter (Vymazal, 2010).

However, the mechanisms that enable CWs to treat polluted water effectively may also be responsible for CH_4 emissions (Kasak et al., 2020; Mander et al., 2023). Besides factors like soil temperature and nutrient availability, the amount of oxygen present, which is closely linked to the water table level in CWs, is a critical factor in generating these gases (Calabrese et al., 2021). Specifically, the production of CH_4 occurs mostly under anaerobic conditions (Yin et al., 2023). Within CWs, underwater ecosystems frequently become anoxic, or oxygen-deficient (Yu et al., 2023a). Specialized microorganisms like methanogens flourish in these anaerobic environments, breaking down organic materials and producing CH_4 as a byproduct in the process (Liu et al., 2021). A considerable amount of this CH_4 escapes into the atmosphere and contributes to global warming, even though part of it is metabolized by other microbes in the CWs (Yu et al., 2023a). As earlier stated, the pace of growth in atmospheric CH_4 concentrations has escalated over the past ten years, with a recorded increase of 17 parts per billion observed in 2021 (*Wetlands and Methane Technical Report*, 2023). If the current increase in atmospheric CH_4 concentration continues, it may become increasingly difficult for the world to reach the targets outlined in the Paris Agreement to prevent disastrous climate change (Nisbet et al., 2019). Thus, while optimizing CWs to effectively treat agricultural runoff or other polluted source water, it is important to minimize CH_4 emissions to combat its effects on global warming.

1. Theoretical background

1.1 Constructed wetland and types

CWs are designed to utilize microbial assemblages, vegetation, and soils found in wetland environments to treat polluted water in a carefully regulated setting while maintaining wetland functions (Wu et al., 2018). CWs used for polluted water treatment can be divided into systems with free-floating, floating-leaved, rooted emergent, and submerged macrophytes, based on the type of prevailing macrophyte. Subsurface flow CW can be categorized based on flow direction (horizontal and vertical), and a further split can be established based on wetland hydrology (free water surface and subsurface systems). While many wetlands have been classified and explored in several studies, this thesis only considers FWS CWs.

FWS CWs are shallow, sealed basins or a series of basins with 20–30 cm of rooting soil and a water depth of 20–40 cm with emergent macrophytes. A large portion of the surface is covered by dense emergent vegetation, typically more than 50%. Naturally occurring species may exist in addition to planted macrophytes (Kadlec, 2008). FWS CWs have a major positive environmental impact by addressing a range of environmental problems, which include the treatment of diffused agricultural pollution from surface waters via nutrient removal, chemical detoxification, and sediment trapping. These wetlands are essential for enhancing water quality (Wei et al., 2021). They achieve this by reducing the amount of nutrients discharged from agricultural fields into the CWs through nutrient absorption by macrophytes which can be harvested (Kasak et al., 2020).

1.2 CH_4 production in CWs

In CWs, the production of CH_4 is a complicated process fueled by a variety of microbial activities (Zhou et al., 2022). Various types of anaerobic microbes, including hydrolytic microorganisms, fermentative microbes, and hydrogen-producing acetogens, break down organic matter from polluted water and plant biomass (Liu et al., 2022). Methanogenic archaea utilize these simpler molecules as substrates to produce CH_4. These methanogens thrive in the anaerobic environment commonly found in the substrate layers of CWs (Wang et al., 2023). Certain CW designs promote these anaerobic conditions. For example, surface flow CWs can develop anaerobic macrozones due to water flow layering within the substrate (Yu et al., 2023a). Anaerobic microbial activity breaks down plant debris, litter, and substrates, ultimately releasing CH_4 and CO_2 into the atmosphere (Gęsicka et al., 2021).

In addition to the role of microorganisms in CH_4 production within CWs, the understanding of plant derived CH_4 emissions is evolving (Yu et al., 2023a). Traditionally, plants were thought to facilitate gas exchange between the soil and the atmosphere (King & Hungria, 2002). However, recent research suggests a more significant role for plants as direct CH_4 emitters under stress conditions (Boros & Keppler, 2019). Studies have observed that plants produce CH_4 as a defense mechanism in response to environmental stressors like UV radiation, high temperatures, physical damage, or disruptions in cytochrome oxidase activity (Schroll et al., 2022). This stress response may involve the overproduction of reactive oxygen species (ROS) such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2). These ROS can exacerbate cellular damage and trigger increased CH_4 production (Wang et al., 2023). A critical knowledge gap exists regarding CH_4 emissions from wetland hydrophyte plants in aerobic (oxygen-rich) environments. This area remains largely unexplored, highlighting the need for further research (Yin et al., 2023).

1.3 CH_4 oxidation and transport pathways in CWs

CH_4 oxidation significantly impacts CH_4 flux throughout its production and transport (Kammann et al., 2001). Aerobic CH_4 oxidation primarily occurs at micro-interfaces where CH_4 and oxygen (O_2) coexist. These interfaces include the rhizosphere (root zone) of plants, the substrate-air, and water-air boundaries, and even within plant tissues (Bonetti et al., 2021; Yu et al., 2023a). The rate of this oxidation process is highly dependent on the concentration of O_2. In contrast, when environments are anaerobic (lacking oxygen), microorganisms employ alternative electron acceptors to oxidize CH_4. These alternative acceptors include sulfate (sulfate-reduction-dependent anaerobic CH_4 oxidation, SAMO), nitrite (nitrite-dependent anaerobic CH_4 oxidation, N-anammox), metal oxides (such as iron (Fe3+) and manganese (Mn4+) dependent anaerobic CH_4 oxidation, metal-AOM), and even direct interspecific electron transfer (Su et al., 2023)

CH_4 can be emitted from wetlands through three processes: diffusion, ebullition (also known as bubble release), and/or plant-mediated transport (Bridgham et al., 2013). Compared to molecular diffusion, ebullition produces three times as many CH_4 fluxes during the direct CH_4 transport process, as seen in Figure 1 (Yu et al., 2023a). While three pathways account for over 70% of total CH_4 emissions, plant-mediated transport is the predominant mode of CH_4 release (Lu et al., 2015). Plant-mediated CH_4 transport mechanisms can be divided into two categories: molecular diffusion and convective transport processes. The primary determinants of molecular diffusion rates are the atmosphere, the inside of the plant organ, and the CH_4 gradient that exists between a plant's roots and above ground sections (Lu et al., 2015).

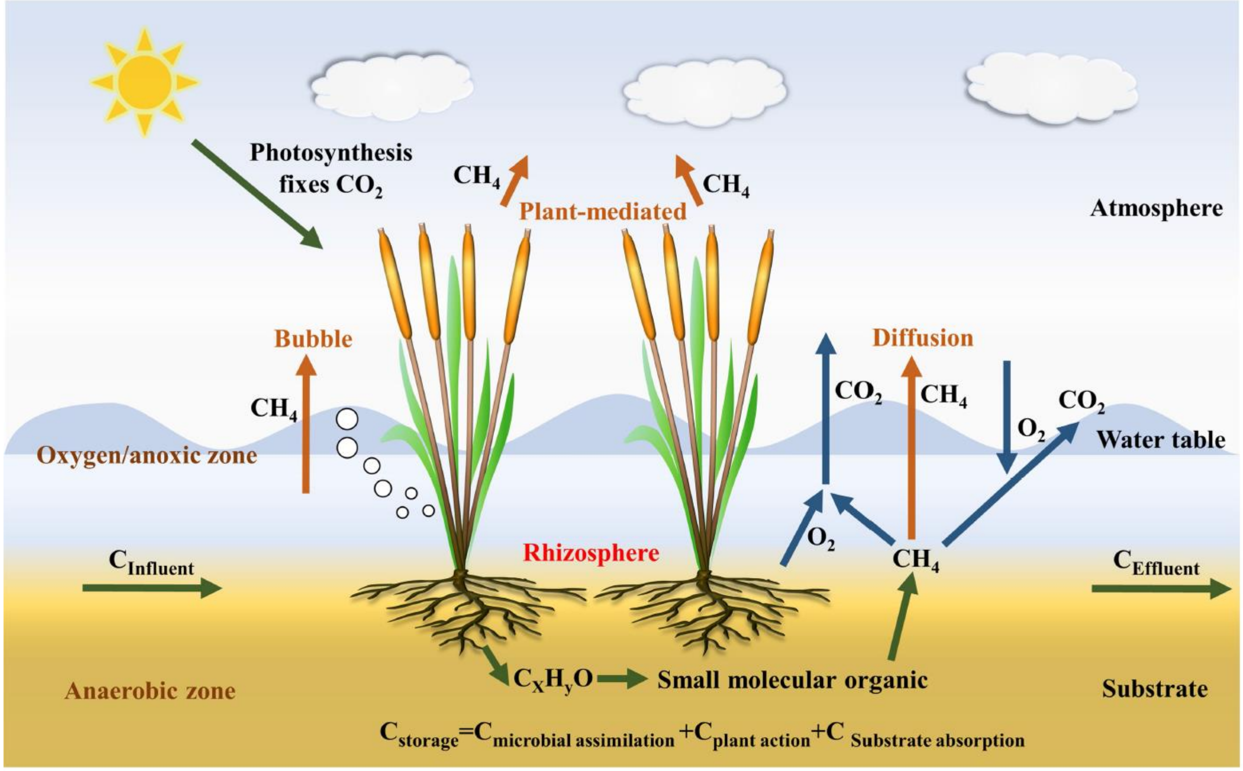

Figure 1: CH_4 production, transport, and oxidation mechanisms in CWs (Source: Yu et al., 2023b).

1.4 Driving mechanism of CH_4 in CWs

1.4.1 Temperature, Oxygen Concentration, and Redox Potential

Temperature plays a significant role in both the production and oxidation of CH_4 in wetlands. The optimal temperature range for methanogenesis falls between 35°C and 40°C (Yin et al., 2023). Low temperatures hinder the activity of methanogenic archaea and fermentative bacteria (Conrad, 2007; Yu et al., 2023a). Studies have shown a somewhat positive correlation between CH_4 production and temperature, assuming sufficient substrate availability (Chang et al., 2020). Beyond influencing overall microbial activity, temperature also affects the dominant methanogenic archaea populations. At warmer temperatures, Methanosarcinaceae, which utilize acetate and H_2/CO_2 as substrates, thrive. However, as temperatures decrease, Methanosaetaceae, specialising in acetate-based CH_4 production, become more dominant (Zhang et al., 2022). In contrast, methanotrophs, responsible for CH4 oxidation, exhibit less temperature sensitivity. Their optimal temperature is around 25°C, but they can function at temperatures ranging from -2°C to 30°C (Zhang et al., 2021). This difference in temperature sensitivity translates to higher CH_4 production in CWs during warm seasons (3.4%–42%) compared to cooler or cold seasons due to increased microbial activity (Shao et al., 2020).

CWs face a challenge in removing organic debris due to the lack of dissolved oxygen (DO). This limitation arises from two factors: prolonged saturation of the CWs media and rapid microbial metabolism within the system (Wang et al., 2022; Yu et al., 2023a). Tidal flow and intermittent aeration strategies are considered the most effective methods for directly influencing CH_4 emissions by managing DO conditions in CWs (Wang et al., 2024). These techniques increase oxygen availability, which serves two purposes: Suppressing CH_4 biological reactions: By introducing more oxygen, these methods can limit the biological processes that generate CH_4 and speed up CH_4 oxidation: Increased oxygen also enhances the activity of methanotrophs, the microorganisms responsible for CH_4 oxidation, leading to faster removal of CH_4 from the system (Wang et al., 2018). Studies suggest that under ideal aeration conditions, intermittent microaeration can achieve significant reductions in CH_4 fluxes, potentially up to 60.7% (Lapham et al., 2022).

The internal oxygen (O_2) level within CWs plays a critical role in both CH_4 production and oxidation. This internal O_2 level is measured by the redox potential (Eh), which also influences the activity of different microbial communities (Liu et al., 2009). Different microbial groups have varying Eh requirements. For instance, aerobic bacteria, responsible for respiration using oxygen, typically thrive at an Eh ranging from +300 mV to +400 mV. Conversely, anaerobic bacteria, which don't require oxygen for respiration, generally become dominant at Eh values below +100 mV (Husson, 2013). Methanogens, located at the end of the respiratory chain, necessitate a highly reducing environment with very low Eh values (optimally around -350 mV). Methanogenic processes, which convert organic matter into CH_4 via anaerobic digestion pathways, typically initiate at Eh values below -200 mV (Kong et al., 2018)

1.4.2 Water level and pH

The water table position within CWs significantly influences the level of anaerobiosis (absence of oxygen) and, consequently, CH_4 emissions (Yu et al., 2023a). A high-water table creates a larger anoxic zone (oxygen-deficient area) by limiting the diffusion rate of atmospheric oxygen O_2 into the substrate. This condition fosters CH_4 production (McInerney & Helton, 2016). In contrast, when the water table sits below the substrate surface, several mechanisms reduce CH_4 emissions. For example, the produced CH_4 can diffuse through the water layer and potentially oxidize before reaching the atmosphere (Bonetti et al., 2021). Also, CH_4 bubbles can rise through the water column, potentially encountering oxygen for oxidation during their ascent (Bhullar et al., 2013). However, a high-water table can lead to increased CH_4 emissions by another route. Under these conditions, most of the produced CH_4 enters the deeper anaerobic layers where plant roots reside. These roots possess aerenchyma, a special tissue that facilitates gas transport (Okiti et al., 2024). Through this pathway, CH_4 can be transported to the atmosphere via the plant shoots, ultimately increasing emissions (Henneberg et al., 2016; Yu et al., 2023; Okiti et al., 2024).

The ability of soils to absorb CH_4 has been observed to decrease with increasing pH, which causes the shift from CH_4 sink to CH_4 source (Qu et al., 2023). This implies that a higher pH encourages the production of CH_4. The mineralization of organic matter, which takes place in anaerobic environments, produces CH_4 through the action of methanogens. Methanogenesis is favourable in areas where the pH is between 6.0 and 8.0 and the oxidation-reduction potential (ORP) is less than -150 mV (Hu et al., 2023). This suggests that CH_4-producing microorganisms thrive best in pH environments that range from slightly acidic to neutral.

2. Aims and objectives.

This thesis aims to study the CH_4 flux dynamics and driving mechanisms of an in-stream FWS CW established for the treatment of diffuse agricultural pollution located at Uhti village, Tartu, Estonia. The specific objectives of this study were.

(1) To quantify the Net flux dynamics from Vända CWs.

(2) To delineate the possible driving mechanisms for these emissions; and

(3) To propose a possible solution for the reduction of these emissions.

3. Materials and methods

3.1 Site description

The Vända FWS CW is in Uhti village in southeast Estonia, in the Vända ditch sub-catchment of the Porijõgi River catchment. Its coordinates are 58.17N, 26.43E. According to Kasak et al. (2018), the catchment area of the Vända FWS CW comprises approximately 62% arable land, 32% natural land, and approximately 8% land used for other purposes. The area has a temperate climate with four seasons that are similar in length. The growing season typically lasts from late April to September. According to data from the nearby Tartu Observatory weather station at Tõravere, this area has an average annual precipitation of 726 mm over 168 days of rainfall. This results in an average air temperature of 6.3°C. The Vända FWS CW consisted of two shallow-water wetlands with a combined size of approximately 4500 m^2. Each wetland has six measurement points (VM1.1-1.6 for the first wetland and VM2.1-2.6 for the second wetland) (Figure 2). Several baffles were built in 2015 to increase the hydraulic efficiency and water retention time of the wetlands. The baffle and riparian zones of both wetlands were lined with stones and geotextiles. Four cattail (*Typha latifolia*) plants were planted in each square meter of the first wetland. Natural encroachment was allowed in the second wetland. Over several years, the first wetland's vegetation noticeably increased by 51%, whereas the second wetland saw a more modest growth of 10.5% (Kill et al., 2018). At present, the entire area is completely covered in vegetation (Mander et al., 2023).

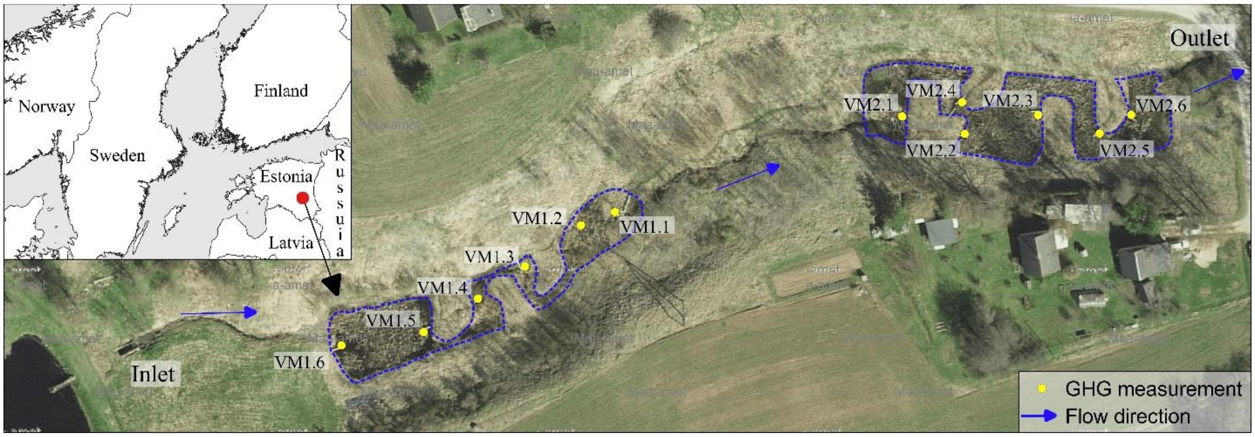

Figure 2: Location map of the study area showing sampling points (VM 1.1 to VM 2.6) for CH_4 and water parameter measurement. (base map source: https://geoportaal.maaamet.ee)

3.1.2 CH_4 gas measurement and calculation

3.1.3 Flux calculation and data quality check

CH_4 measurements were carried out biweekly using the LI-7810 gas analyzer (LI-COR, Lincoln, NE, USA) from October 2022 to December 2023 (15 months). The biweekly CH_4 gas concentration measurements were carried out for five minutes at each measurement point, and the measurements were taken from 12 sampling points on both wetlands (Figure 2) using an opaque chamber of area $0.196m^2$ and volume $0.065m^3$ painted white to avoid heating during the gas measurement. The truncated conical chamber was connected via tubing to the CH4 gas analyzer equipped with a fan to promote mixing. CH_4 fluxes were then calculated from the measured gas concentration using the following equation which is in line with previous research (Ranniku et al., 2023; Okiti et al., 2024), (equation (1)):

$$\text{Flux } [\mu g\ m^{-2}\ hr^{-1}] = \left[\frac{P}{R.T}\right] * \left[\frac{dG}{dt}\right] * \left[\frac{V}{A}\right] * M \quad (1)$$

Where P = air pressure (Pa), R = gas constant (8.314), T= Average chamber temperature during measurement, dG/dt = slope of the flux measurement (ppm s−1), V= measurement chamber volume (m^3), A = Surface area covered by the chamber (m^2), and M = molecular mass of the gas (CH_4 = 16.04246). The quality of the chamber measurement was confirmed by assessing the adjusted R^2 value from the linear regression. Only flux measurements with a coefficient of determination (R^2) of 0.9 or higher were considered suitable for additional analysis in calculating diffusive fluxes (Figure 3A). However, for the net fluxes, which in some cases include bubbles or ebullition, we utilized R^2 values that are less than 0.9, as shown, for example, in Figure 3B.

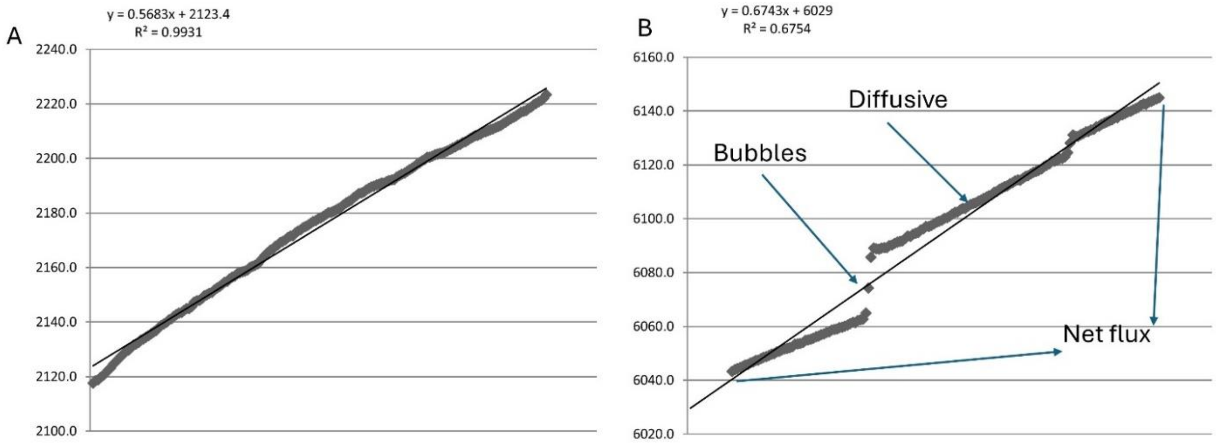

Figure 3: Example of flux calculation plots with R^2 >0.9 (A) and R^2 < 0.9 (B).

3.1.4 Water parameters

Biweekly field measurements were also conducted using the YSI ProDSS (YSI Inc., Yellow Springs, OH, USA) measuring device. This instrument measured water quality parameters including turbidity, water temperature, pH, oxygen concentration, conductivity, and oxidative reduction potential. Once the sensor cable was carefully dipped into the water, the YSI ProDSS took about 5 minutes to measure the chosen parameters and display the results on its screen.

Figure 4: Author in the field, measuring CH_4 gas and water parameters using the LI-7810 trace gas analyzer (left) and YSI ProDSS (right).

3.1.5 Data Structure and Statistical Analysis

3.1.6 Data structure

The data available for analysis includes:

Fifteen (15) months (October 2022 to December 2024) of gas emission measurements.

Fifteen (15) months (October 2022 to December 2024) of other wetlands water parameters such as turbidity, electrical conductivity, and dissolved oxygen concentration, among others.

The author of the current thesis participated in data collection and data analyses and conducted all analyses and graphs.

3.1.7 Statistical Analysis

The normality of the variables was assessed using the Shapiro-Wilk and Anderson-Darling tests. Due to deviations from the normal distribution, nonparametric tests were employed. Data analysis and figures were generated using R 4.3.3 (Posit Team, 2024), with statistical significance set at $p < 0.05$ for all tests. Tidyverse package (Wickham et al., 2019) was used for the visualization while the corrplot package (Wei & Simko, 2021) was used for the correlation analysis.

3.2 Results

This section presents the results of the spatial analyses relevant to the objectives of this master's thesis. To visually represent the CH_4 emitted from the first and second wetlands, 12 measurement locations (VM1.1-VM1.6 for the first wetland and VM2.1-VM2.6 for the second wetland; Figure 2) were sampled from October 2022 to December 2023. The Wetlands were examined for possible spatial variations and seasonal fluctuations in CH_4 flux dynamics. From the spatial analyses, it can be observed that the emissions from all sampling points varied across the two wetlands. Specifically, in the first wetland CH_4 emissions in most of the sampling points vary between 5 $\mu g\ m^{-2}\ hr^{-1}$ (during the winter months) to about 4,000 $\mu g\ m^{-2}\ hr^{-1}$ (during the summer months) except in VM 1.2 which showed an exceptionally high emission level exceeding 80,000 $\mu g\ m^{-2}\ hr^{-1}$ as seen in Figure 5

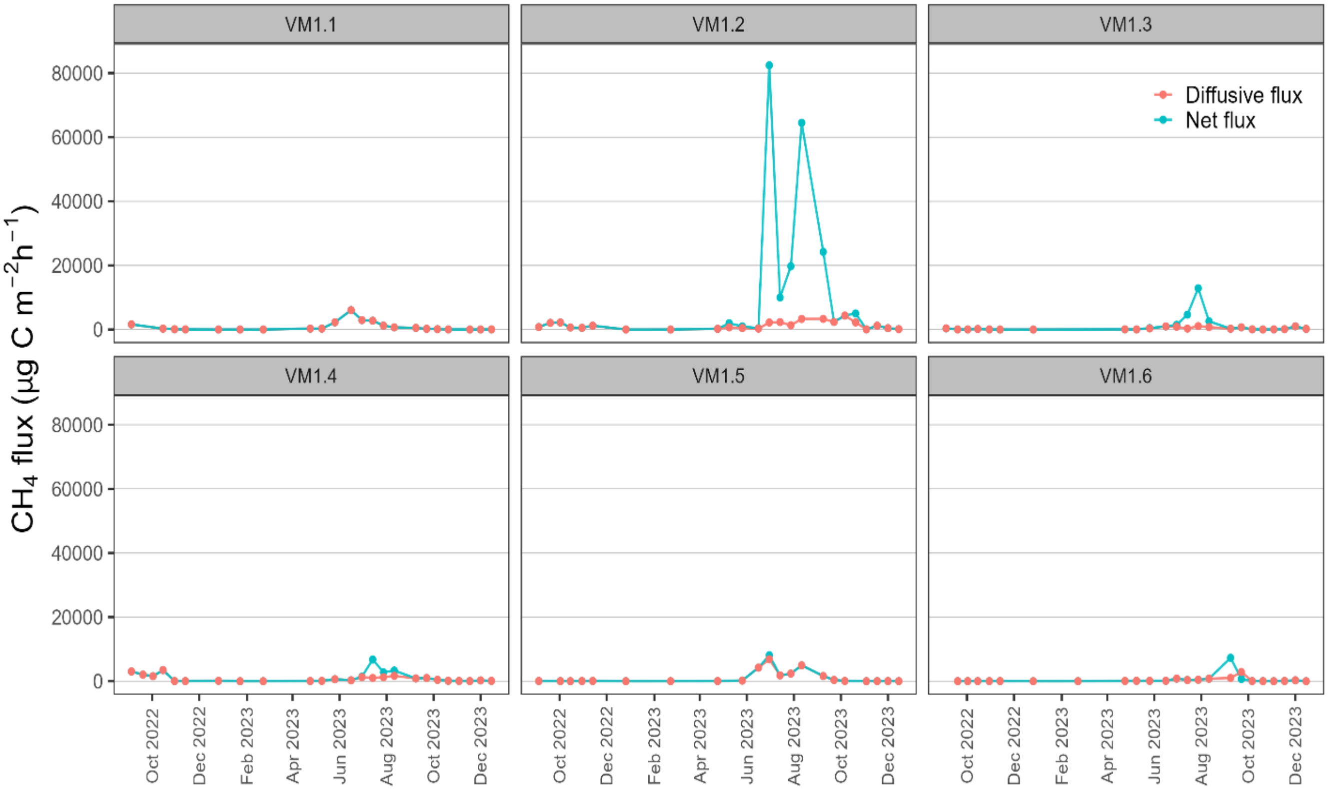

Figure 5: Line graphs showing CH_4 emissions levels across the six sampling points within the first wetland.

Also, some of the measurement points in the second wetland showed high CH_4 emissions. For example, VM 2.1 and VM 2.6 show above 40,000 µg m^{-2} hr^{-1} and VM 2.5 shows emissions of about 70,000 µg m^{-2} hr^{-1}. However, VM 2.4 shows emissions of about 10,000 µg m^{-2} hr^{-1} as seen in Figure 6

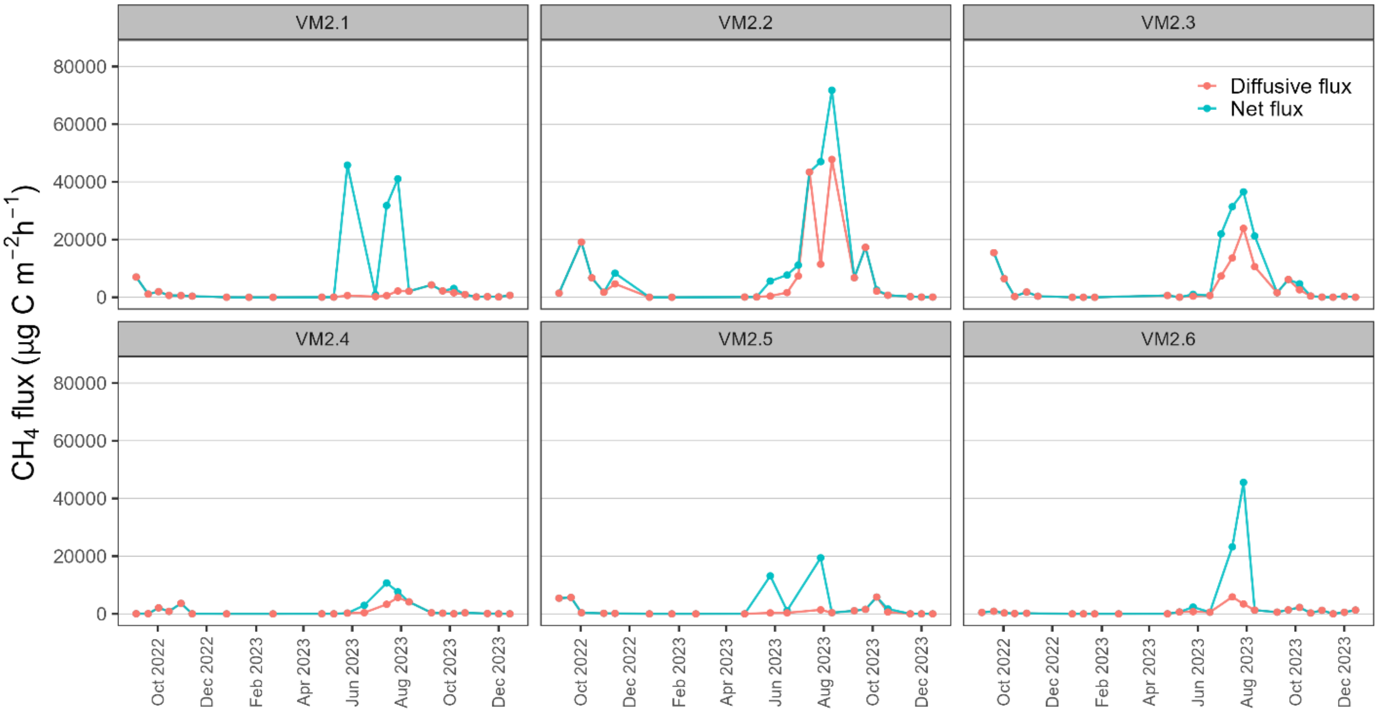

Figure 6|: Line graphs showing CH_4 emissions levels across the six sampling points within the second wetland.

To compare overall CH_4 emissions across the Wetlands, the average emission for all sampling points in each wetland was calculated. The result of the analysis showed that the second wetland showed the highest diffusive CH_4 flux emission of median 420.8 µg m^{-2} hr^{-1} (2657.2 µg m^{-2} hr^{-1} ± 523.7 µg m^{-2} hr^{-1} mean, ± standard error (SE)) with a peak emission of about 12,000 µg m^{-2} hr^{-1} when compared to the first wetland which shows a peak emission of about 2,000 µg m^{-2} hr^{-1}, and median of 218.9 µg m^{-2} hr^{-1} (784.2 µg m^{-2} hr^{-1} ± 99.2 µg m^{-2} hr^{-1}). Generally, both wetlands displayed overall high net CH_4 average emissions. For example, the second wetland showed higher emission with a median of 619.2 µg m^{-2} hr^{-1}, (5306.1 µg m^{-2} hr^{-1} ± 936.5 µg m^{-2} hr^{-1}) and a peak emission of about 35,000 µg m^{-2} hr^{-1} compare to the first wetland which showed a median of 218.9 µg m^{-2} hr^{-1} (2424.4 µg m^{-2} hr^{-1} ±743.9 µg m^{-2} hr^{-1}) reaching a peak of above 15,000 µg m^{-2} hr^{-1} (Figure 7).

Figure 7 shows that there is a difference between the patterns in overall CH_4 emissions between measuring points across the various months. For instance, in the first wetland, CH_4 emission shows a value of about 1000 µg m^{-2} hr^{-1} in September 2022 and rises to over 15,000 µg m⁻² hr⁻¹ in July 2023. However, these emissions then drop to below 5 µg m⁻² hr⁻¹ in November 2023. In contrast, the CH_4 emissions vary in different measuring points across different months in the second wetland. For instance, in September 2022, CH_4 emissions were about 3,000 µg m⁻² hr⁻¹ but rose to 35,000 µg m⁻² hr⁻¹ in August 2023. However, there was a similar trend in the winter months, with CH_4 emissions reaching a value below 5 µg m⁻² hr⁻¹ in November 2023 (Figure 7).

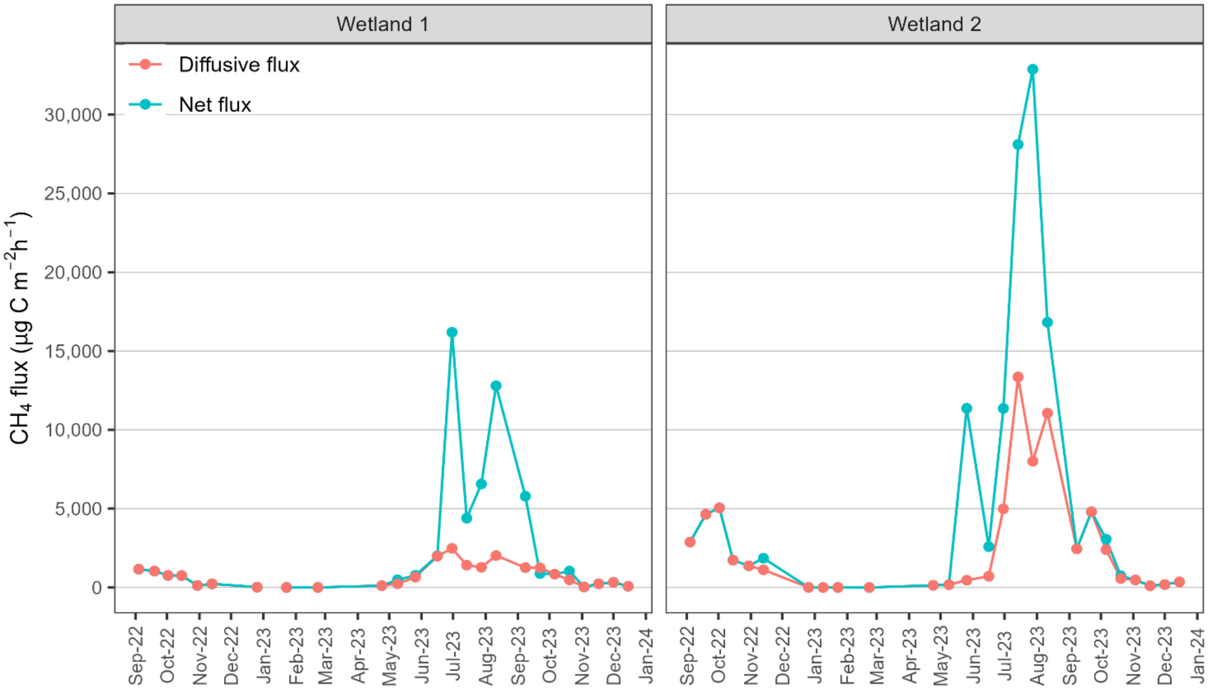

Figure 7: Line graphs showing variation in average CH_4 emissions across different months of the measurement period from the first and second Wetland.

A comparative analysis of the seasonal variations in CH_4 emissions between the wetlands was conducted to determine seasonal fluctuations in CH_4 emissions, as shown in Figure 8. The result indicated that in the summer months (June - August). The first and second wetlands show the highest overall CH_4 emission rates with a median of: 2666.8 µg m⁻² hr⁻¹ (8393.3µg m⁻² hr⁻¹±3351.6 µg m⁻² hr⁻¹) in the first wetland and a median of 11150.8 µg m⁻² hr⁻¹ (19430.7 µg m⁻² hr⁻¹ ±3563.4 µg m⁻² hr⁻¹) in the second wetland. During the fall month (September to November) both wetlands displayed a moderate increase in CH_4 emissions, with a median of: 103.5 µg m⁻² hr⁻¹, (1097.1 µg m⁻² hr⁻¹ ± 375.5 µg m⁻² hr⁻¹) in the first wetland and a median of 880.5 µg m⁻²

hr^{-1} (2501.5 μg m^{-2} hr^{-1} ± 465.7 μg m^{-2} hr^{-1}) in the second wetland. However in Spring (march to May) and Winter months (December to February), both wetlands exhibited low CH_4 emissions with a median of 205.7 μg m^{-2} hr^{-1}, (457.9 μg m^{-2} hr^{-1} ± 157.9 μg m^{-2} hr^{-1}) during the spring in first wetland and median of 126.2 μg m^{-2} hr^{-1} (4114.6 μg m^{-2} hr^{-1} ± 2650.3 μg m^{-2} hr^{-1}.) in the second wetland. During the winter, the overall CH_4 emission in both wetlands was lowest with a median of 4.9 μg m^{-2} hr^{-1}, (103.5 μg m^{-2} hr^{-1} ±36.1 μg m^{-2} hr^{-1}) in the first wetland and a median of 4.9 μg m^{-2} hr^{-1} (114.1 μg m^{-2} hr^{-1} ± 47.4 μg m^{-2} hr^{-1}) in the second wetland (Figure 8).

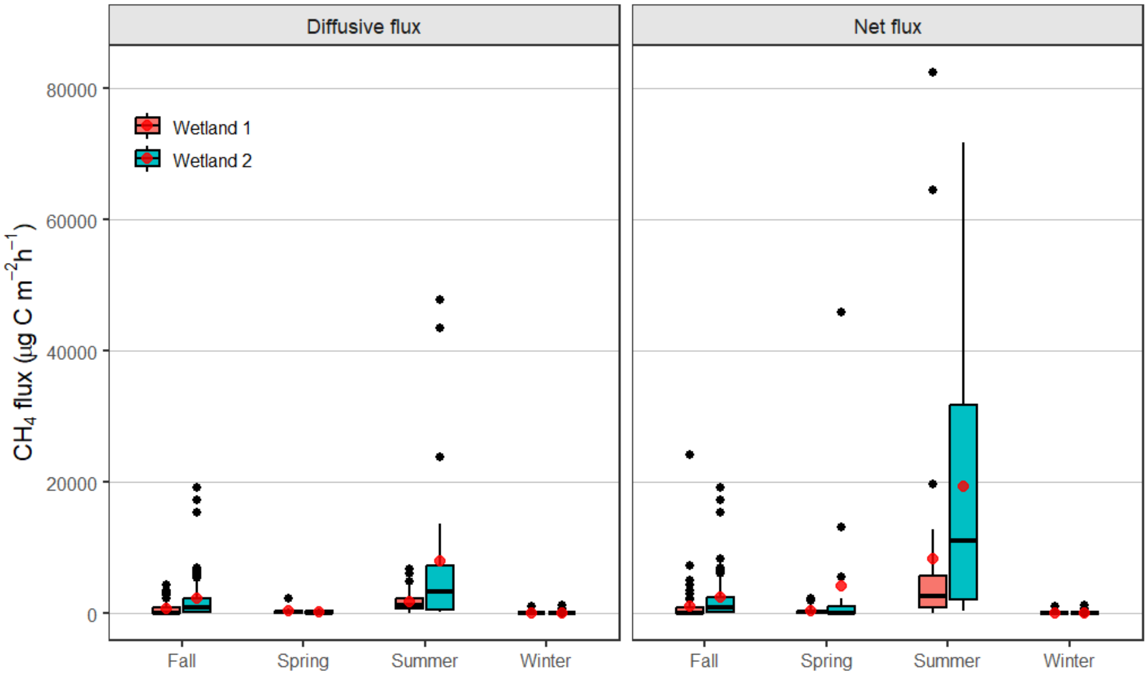

Figure 8: Box plot showing the level of CH_4. emissions in different seasons during the study period. Solid horizontal lines show medians; boxes are the interquartile range, and whiskers are the minimum-maximum values. Black points denote outliers. Pink dots denote average.

3.2.1 The relationship between measured parameters and CH_4 flux

The relationship between CH_4 emissions and various measured parameters, which include water temperature, soil temperature, turbidity, oxidation-reduction potential, pH, conductivity, and water level in both wetlands, are shown in the Figures below.

Figures 9 to 13 show a similar correlation between water temperature, conductivity, oxygen reduction potential (ORP), pH, turbidity and CH_4 emissions in both wetlands. Higher values of these parameters are associated with an increase in CH_4 emissions. However, the average temperature, conductivity, ORP, pH, and turbidity (10.1°C, 465.3, 140.2 mV, 7.3 and 19.1, respectively) in the second wetland is higher than the temperature, conductivity, ORP, pH, and turbidity (9.7 °C, 396.8, 129.1 Mv, 7.3, and 22.6, respectively) in the first wetland except turbidity with a higher average value in first wetland.

Figure 9: Line graph showing a similar pattern between average net CH_4 emission and water temperature in both wetlands.

Figure 10: Line graph showing a similar pattern between average net CH_4 emission and conductivity in both wetlands.

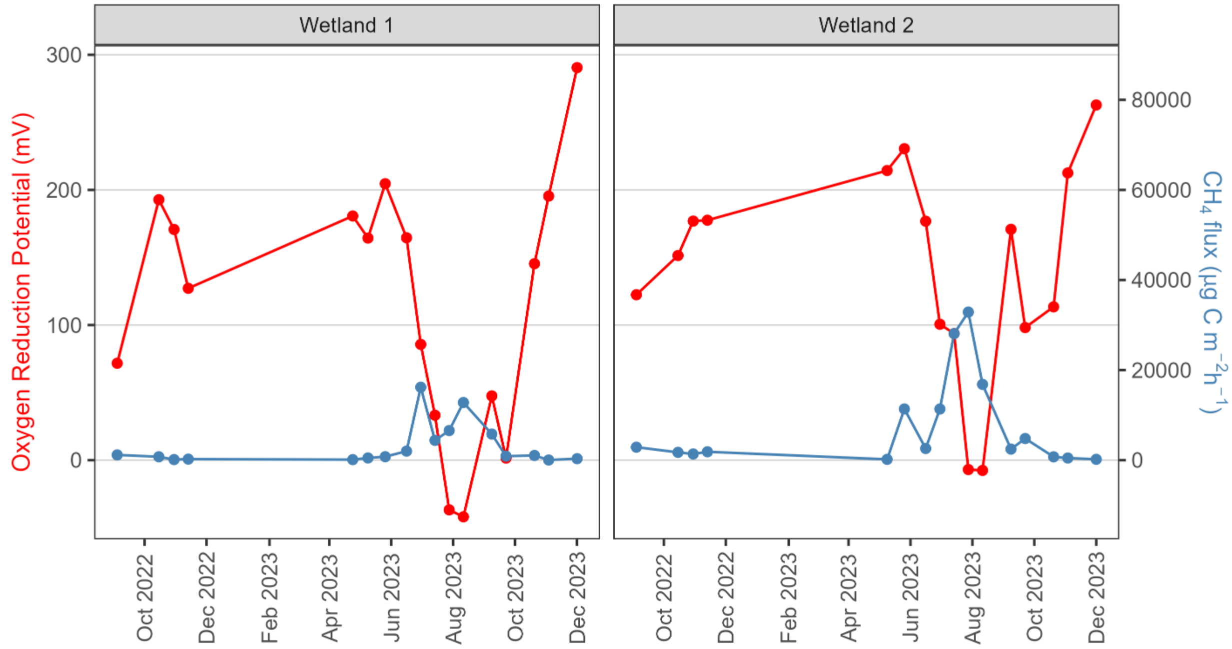

Figure 11: Line graph showing a similar pattern between average net CH_4 emission and ORP in both wetlands.

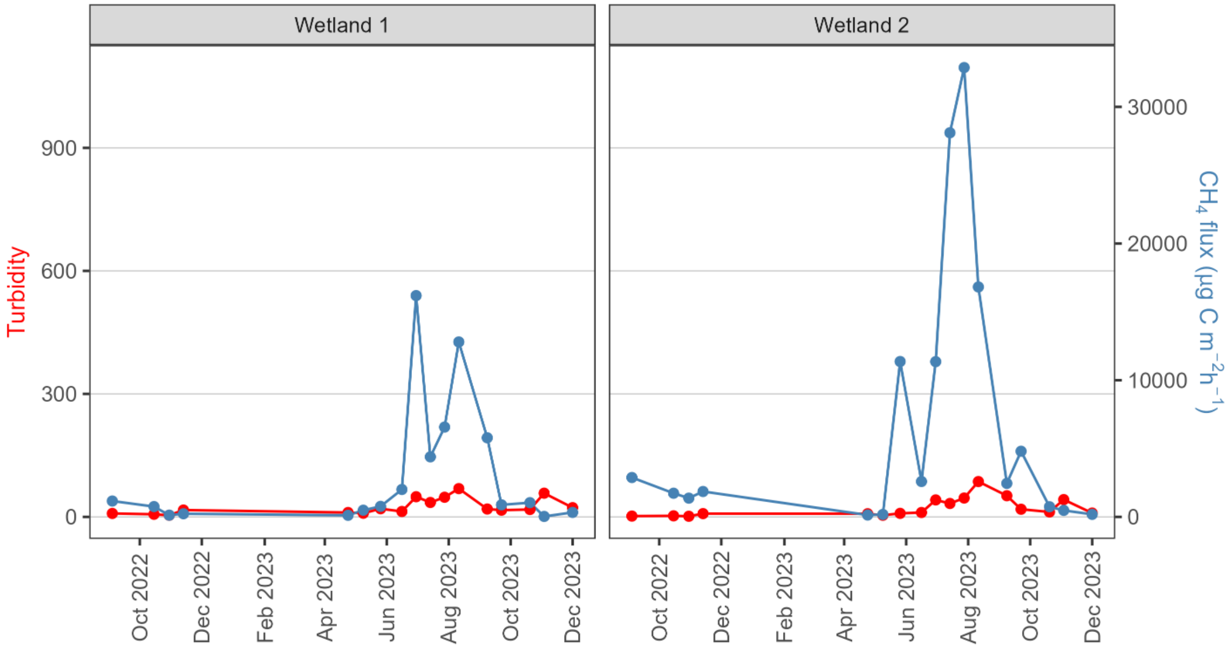

Figure 12: Line graph showing a similar pattern between average net CH$_4$ emission and turbidity in both wetlands.

Figure 13: Line graph showing a similar pattern between average net CH$_4$ emission and pH in both wetlands.

Conversely, the oxygen concentration and water level showed a dissimilar pattern with CH_4 emissions in both wetlands, as shown in Figures 14 and 15. However, the average oxygen and water level (7.5 mg/l and 17.4 cm) in the second wetland is higher than the oxygen and water level (8.8 mg/l and 16.2 cm) in the first wetland.

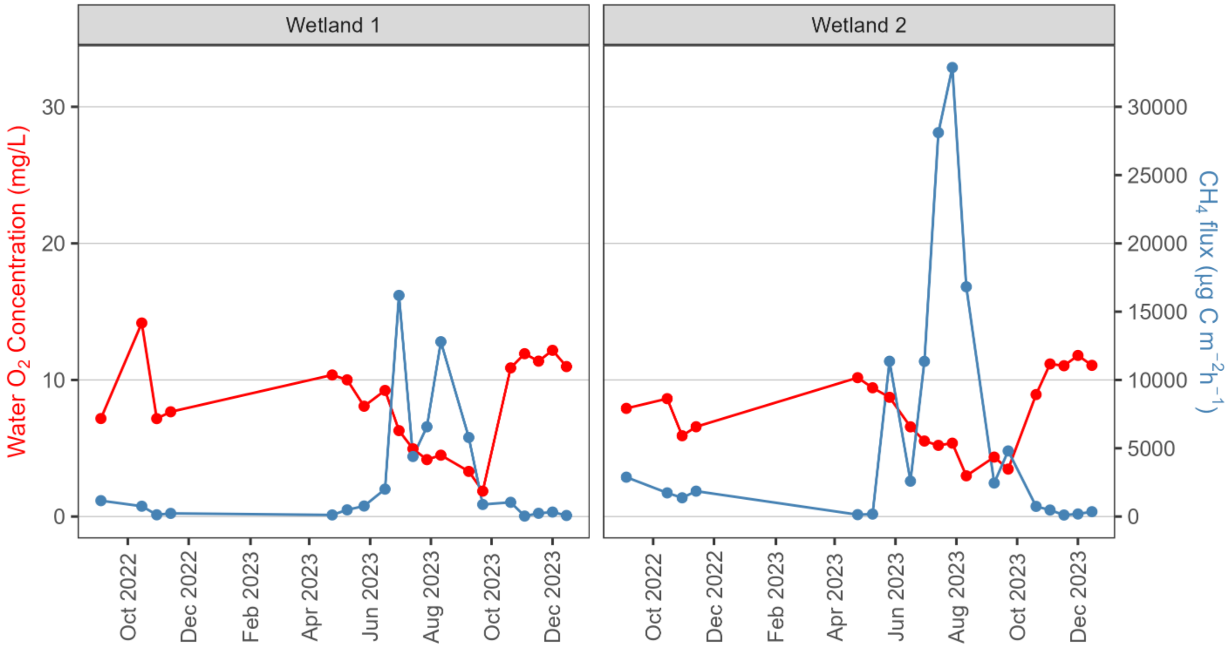

Figure 14: Line graph showing a dissimilar pattern between average net CH_4 emission and oxygen concentration in both wetlands.

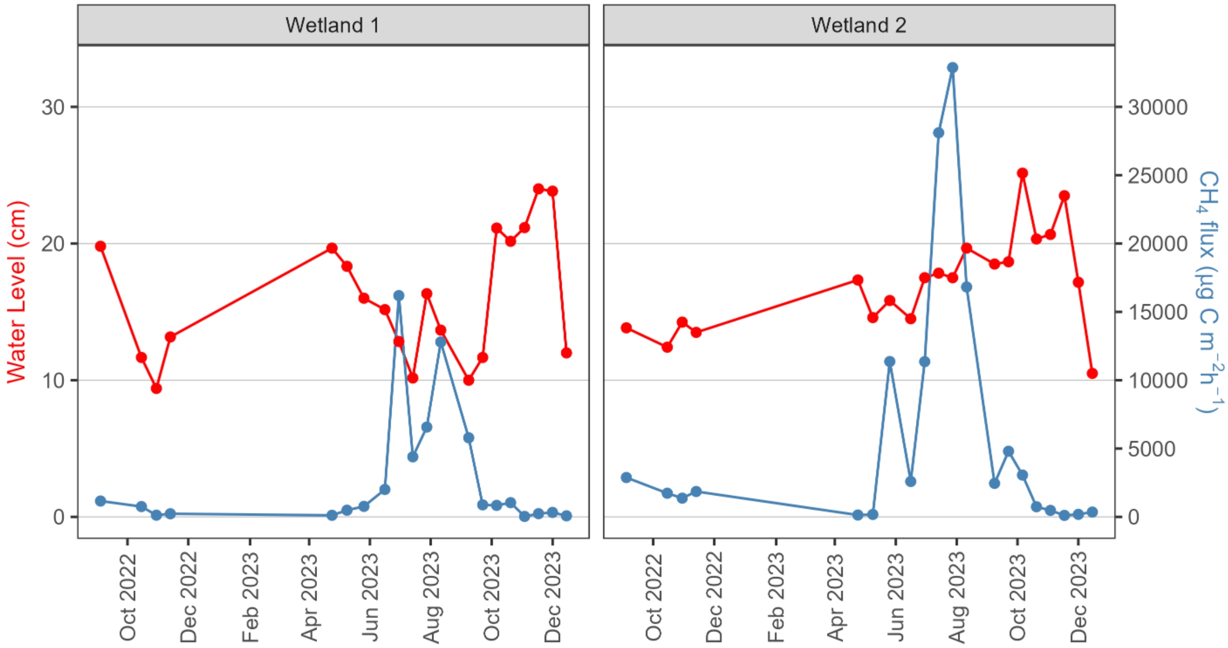

Figure 15: Line graphs showing a dissimilar pattern between average net CH_4 emission and water level in both wetlands.

To investigate the influence of each measured parameter on CH_4 emission in both wetlands, a Spearman's rank correlation analysis was conducted. This analysis assessed the strength of the relationships between CH_4 emission and various measured parameters. In the first wetland, Spearman's rank correlation analysis revealed that several measured parameters, including dissolved oxygen (O_2), pH, oxidation-reduction potential (ORP), and conductivity, exhibited statistically significant correlations (<0.001) with CH_4 emission when compared to other parameters (Figure 16).

For the second wetland, Spearman's rank correlation analysis revealed that conductivity, dissolved oxygen, ORP, and water temperature are key factors influencing CH_4 emissions. From the analysis, conductivity, ORP, and water temperature emerged as the most significant factors in methane emission, with p-values of <0.001, respectively. Also, turbidity and dissolved oxygen had p-values of <0.05, respectively. Like the first wetland, pH and water levels showed no significant correlation with methane emissions (Figure 17).

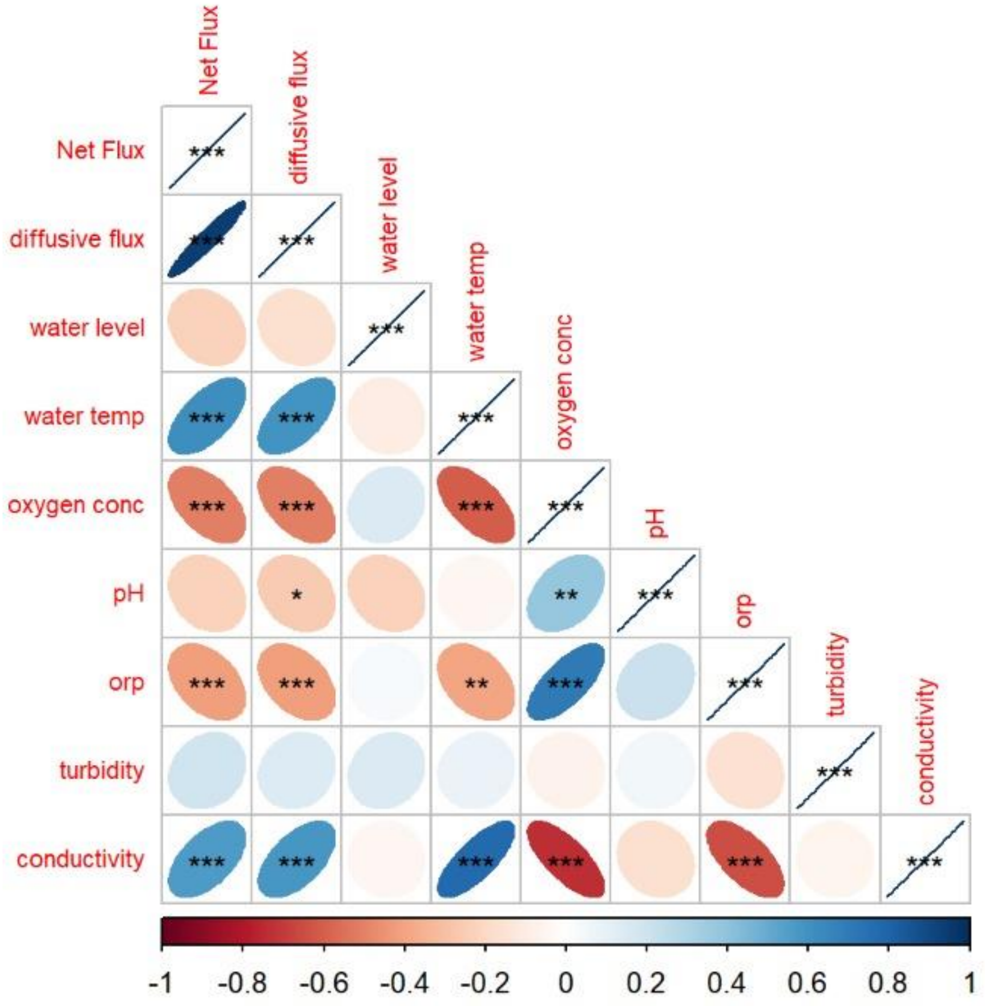

Figure 16: Spearman correlation heatmap showing the relationship between methane emissions and measured parameters in the first wetland. Colour coding represents the Spearman correlation coefficient (ρ). *, **, *** represent the level of significance for p values <0.05, <0.01, <0.001 respectively.

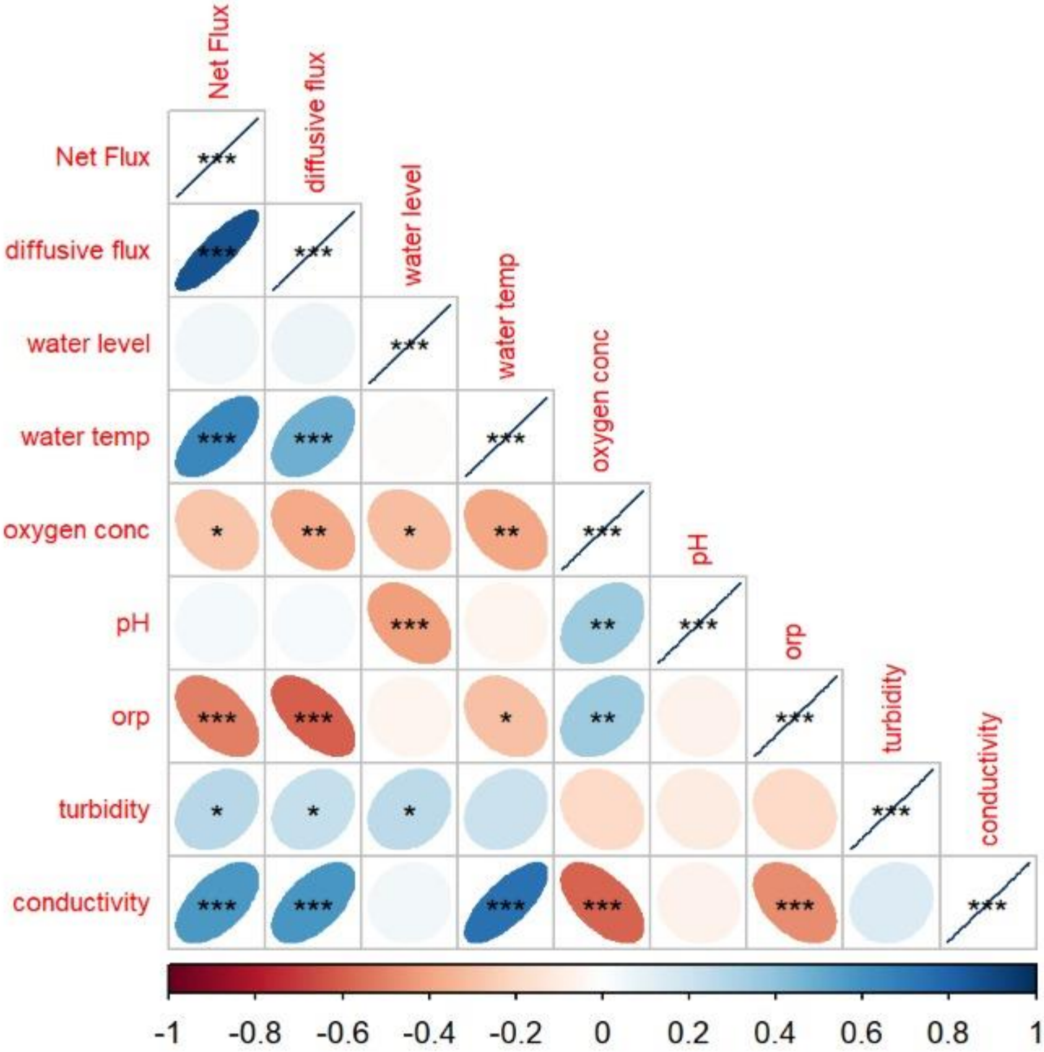

Figure 17: Spearman correlation heatmap showing the relationship between methane emissions and measured parameters in the second wetland. Colour coding represents the Spearman correlation coefficient (ρ). *, **, *** represent the level of significance for p values <0.05, <0.01, <0.001 respectively.

3.3. Discussion

3.3.1 CH_4 flux dynamics in studied wetlands

CH_4, a trace gas, primarily of natural biological processes, influences atmospheric chemistry and radiative transfer processes. CH_4 is a highly effective greenhouse gas, coming in second only to CO_2 in its capacity to trap heat in the atmosphere (Myhre et al., 2013). The study result showed that Vända CWs emit CH_4. This finding is in line with Nisbet et al. (2014) who noted that the wetland ecosystem accounts for one-third of the global annual CH_4 emissions, making it the largest natural source of CH_4 released into the atmosphere. Specifically, Tan et al., (2020) reported that CWs can also significantly contribute to the total CH_4 emissions. The study results also indicate significant variability in CH_4 emissions in Vända Wetlands, with higher CH_4 emissions observed in the second wetland compared to the first wetland. This finding aligns with Zhu et al. (2013), who highlighted substantial variations in CH_4 release across different locations.

3.3.2 The relationship between measured parameters and CH_4 emissions

The current study shows that the key drivers of CH_4 emissions include dissolved oxygen, pH, oxidation-reduction potential, water temperature, and conductivity. This is in line with previous studies, which noted that temperature, oxygen concentration, and pH play a critical role in CH_4 emission rates (Chang et al., 2021; Liu, 2021.). For example, in the second wetland, where dissolved oxygen levels are lower and water temperature is higher compared to the first wetland, anaerobic conditions are predominant, promoting higher emission of CH_4. This suggests that microbial activity in wetlands is primarily influenced by factors such as dissolved oxygen availability and water temperature. The lower dissolved oxygen levels create an environment conducive to CH_4 emission, while the higher water temperature accelerates microbial metabolic rates, further enhancing CH_4 emissions (Wallenius et al., 2021). The study result showed a seasonal fluctuation in the emission of CH_4 with a higher emission of CH_4 during summer as against fall, spring, and winter. This finding is consistent with patterns shown in wetland research, wherein the synthesis of CH_4 is stimulated by warmer temperatures through microbial activity (Zhang et al., 2022). Similarly, some research has suggested clear seasonal patterns of CH_4 fluxes that are associated with changes in season (Yu et al., 2023; Mander et al., 2023). Thus, these could be a possible reason for the observed higher CH_4 flux in the summer months compared to other months in our studies. During the summer months, soil and water temperatures are higher, and higher temperatures accelerate the metabolic rates of methanogenic bacteria, leading to increased CH_4 emissions (Yu et al., 2023a). Also, during summer months, when microbial activity is heightened, the demand for oxygen by aerobic organisms increases, further reducing dissolved oxygen levels and promoting anaerobic conditions (Cyprowski et al., 2018). This enhanced anaerobic environment during summer facilitates CH_4 emission, which may be a contributing factor to the observed seasonal variation in our studies.

Another factor that can account for the differences in the emissions between these wetlands is the level of electrical conductivity. For instance, the higher conductivity in wetland two indicates a greater presence of dissolved ions, which may result from increased organic matter decomposition. This decomposition might have provided additional substrates for methanogenic bacteria, leading to an increase in CH_4 emissions in the second wetland. Moreover, the higher turbidity in the second wetland suggests greater levels of suspended particles and organic matter, which can provide microhabitats for CH_4-producing microbes. Changes in microbial communities due to turbidity alterations may further enhance CH_4 emission rates (Lu et al., 2015). pH has also been seen as a driving mechanism in CWs (Chang et al., 2021; Liu, 2021). However, we did not find a clear relationship in our studies. Thus, there are distinct factors driving CH_4 emissions in Vända Wetlands. The relative importance of these factors in CH_4 emission varied between the first and second wetland, with dissolved oxygen, conductivity, and water temperature playing crucial roles in regulating CH_4 emission in the first wetland, while conductivity, ORP, and water temperature are the most significant factors in CH_4 emission in the second wetland.

3.3.3 Recommendation to reduce CH_4 flux.

Based on the findings from this study, we can recommend the harvesting of plant biomass. Harvesting of plant biomass can effectively minimize CH_4 emissions in CWs by influencing the decomposition process (Xie et al., 2022). Therefore, the removal of excess plant biomass from CWs can help managers control several key drivers of CH_4 emissions, including dissolved oxygen, pH, oxidation-reduction potential, and conductivity. This strategic approach not only reduces CH_4 emissions but also capitalizes on the renewable resources of plant biomass, promoting more environmentally friendly decomposition processes within CWs. Given that water temperature has been identified as one of the key drivers of CH_4 emissions in CWs, we recommend implementing shading or planting more trees as a viable strategy to reduce the water temperature, which in turn can help minimize CH_4 emissions since microbial activities become more active in higher temperature as discussed earlier. Vegetation, especially harvesting plants with aerenchyma (e.g. *Phragmites australis, Typha latifolia*), can reduce CH_4 emissions and influence microclimate (Kutzbach et al., 2004). This strategy not only addresses elevated water temperatures but also enhances habitat and ecosystem resilience, thus enhancing the overall ecosystem.

Finally, to effectively reduce CH_4 emissions in the Vända wetlands, we recommend the strategic implementation of aeration systems, such as intermittent micro-aeration (Fang et al., 2022). Aeration enhances oxygen transfer efficiency, which in turn plays an important role in controlling CH_4 emissions (Yang et al., 2023). Therefore, creating aerobic conditions through intermittent micro-aeration helps reduce methanogenic activity, thereby reducing the release of CH_4. This approach not only promotes sustainable diffuse agricultural treatment practices but is also in line with global efforts to reduce greenhouse gas emissions and combat climate change.

Summary

Vända CWs are used to treat agricultural runoffs. However, while effective in this role, it can also emit CH_4 due to the anaerobic decomposition of organic matter. To effectively track the emission levels of these gases, objective, independent research is crucial to identify patterns and evaluate the effectiveness of mitigation strategies. This highlights the importance of this thesis, which seeks to study the CH_4 flux dynamics and driving mechanisms of the in-stream Vända CW established for treating diffuse agricultural pollution located in Uhti village, Tartu, Estonia.

To measure the CH_4 emissions, we utilized the LI-7810 gas analyzer (LI-COR, Lincoln, NE, USA) with a closed chamber on a bi-weekly basis from October 2022 to December 2023. Also, water parameters such as dissolved O_2, pH, ORP, water temperature, turbidity, and conductivity were measured on a biweekly basis using the YSI ProDSS (YSI Inc., Yellow Springs, OH, USA) measuring device. The CH_4 flux results show that the CWs emit high CH_4 fluxes, with emission reaching a peak of about 80000 $\mu g\ m^{-2}\ hr^{-1}$ at one sampling point. However, there was a clear variation between the summer month and other months, as maximum emissions were recorded during the summer month when the temperature was at its peak compared to other months, with the least emissions observed during the winter months. Spearman's rank correlation analysis was further utilized to investigate the influence of the measured water parameters on CH_4 emission in the CWs. This analysis assessed the strength of the relationships between CH_4 emission and various measured parameters. The analysis revealed that several measured parameters, including dissolved O_2, pH, ORP, water temperature, turbidity, and conductivity, exhibited statistically significant correlations ($p < 0.05$) with CH_4 emission. Thus, playing a major role as a possible driver of the methane flux.

Based on the findings from this study, we can recommend harvesting plant biomass. Harvesting plant biomass can effectively minimize CH_4 emissions in CWs by influencing the decomposition process because removing excess plant biomass from CWs can help control several key drivers of CH_4 emissions, including dissolved oxygen, pH, oxidation-reduction potential, and conductivity. We also recommend the strategic implementation of aeration systems, such as intermittent micro-aeration. Aeration enhances oxygen transfer efficiency, which in turn plays an important role in controlling CH_4 emission. Therefore, creating aerobic conditions through intermittent micro-aeration helps reduce methanogenic activity, thereby reducing the release of

CH_4. This approach not only promotes sustainable diffuse agricultural treatment practices but is also in line with global efforts to reduce greenhouse gas emissions and combat climate change.

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